DOCKET NO. 13131-0370 (44378-300773)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Cham et al.	)	
	)	Art Unit: **1648**
Serial No. **10/873,015**	)	
	)	Examiner: **STACY B. CHEN**
Filed: **JUNE 21, 2004**	)	
	)	
For: **MODIFIED VIRAL PARTICLES WITH IMMUNOGENIC PROPERTIES AND REDUCED LIPID CONTENT USEFUL FOR TREATING AND PREVENTING INFECTIOUS DISEASES**	)	

DECLARATION OF DR. MOIZ KITABWALLA UNDER 37 C.F.R. §1.132

I, Moiz Kitabwalla, Ph.D., do hereby declare:

1. I am an expert in the field of virology. I am currently the Senior Manager of Viral Programs at Lipid Sciences, Inc. (Pleasanton, CA). I earned a B.Sc. degree (*summa cum laude*) in 1995 at the University of Wisconsin-Eau Claire. I earned a Ph.D. degree in Molecular Biology in 2001 at the University of Wisconsin-Madison. My *curriculum vitae* is enclosed (Exhibit A). I published over 13 papers in the field of Virology and Immunology, specifically, HIV and SIV research. The list of the publications is enclosed (Exhibit B).

2. I have read the above-referenced patent application ("present application") and the pending claims. I have analyzed the reference cited by the Examiner in the above-referenced patent application: U.S. Patent No. 6,136,321 ("Barrett"), and the rejections of Claims 1-5, 7-11, 14, 16, 18-21, 23, 27 and 28 under 35 U.S.C. §102(b) as anticipated by Barrett in the Final Office Action mailed September 7, 2007.

3. As one of ordinary skill in the art, I declare that the experimental results provided herein demonstrate that partially delipidated human immunodeficiency viral particles obtained by delipidation with an organic solvent that is not a detergent or a surfactant, for example, diisopropyl ether (DIPE) ("partially delipidated HIV particles"), are structurally different from the delipidated human immunodeficiency virus (HIV) obtained by a detergent treatment method disclosed in Barrett ("detergent-treated HIV").

4. As one of ordinary skill in the art, I declare that the experimental results provided herein demonstrate that partially delipidated HIV particles possess unexpected advantages as compared to detergent-treated HIV.

5. Experimental procedures used for obtaining partially delipidated HIV particles and detergent-treated HIV were as follows. HIV ("stock HIV"), namely, purified HIV-IIIB (directly pelleted, catalog# 10-124-000, lot# 2K0014B ("the lot")) was obtained from ABI Inc. The Product Specification for the lot is attached in Exhibit C. The 50% Tissue Culture Infective Dose ($TCID_{50}$) content of this lot was $10^{7.33}$ $TCID_{50}$/mL.

Detergent-treated HIV was obtained according to a process disclosed in Barrett, for example, in column 7, lines 15-25. Briefly, Tween 80 was added to stock HIV to a final concentration of 11% to 1 mL of the solution of stock HIV. The sample was then incubated for 1 hr at 26°C, diluted to 8.9 mL in 1X phosphate buffered saline (PBS) after incubation, and the detergent-treated HIV was pelleted by centrifugation at 400,000g for 10 min at 4°C. The pelleted detergent-treated HIV was resuspended in 0.6 mL 1X PBS, and assayed using Western blot analysis, p24 Enzyme-Linked ImmunoSorbent Assay (ELISA), and electron microscopy (EM), according to conventional procedures.

Partially delipidated HIV particles were obtained, briefly, as follows. DIPE was added to stock HIV to a final concentration 1.8% v/v. The sample was then mixed by end-over-end rotation at 70% efficiency for 20 min at room temperature, centrifuged for 2 min at 2000 rpm at room temperature, and the supernatant was passed through a mini charcoal tube, primed with PBS, at a rate of 1 drop/second, with 0.5 ml of PBS. The resulting sample of partially delipidated HIV particles was assayed using Western blot analysis, p24 Enzyme-Linked ImmunoSorbent Assay (ELISA), and electron microscopy (EM), according to conventional procedures.

6. Experimental results provided herein show that detergent-treated HIV contains substantially less HIV p24 Capsid Protein ("p24") as compared to partially delipidated HIV particles. In reference to Figure 1, detergent-treated HIV, partially delipidated HIV particles, and control untreated HIV stock ("control") samples were assayed for p24 content using Western

blot analysis. Equal amounts of protein (10 μg) from each sample were loaded per each lane in the gel analyzed by western blot. Figure 1A illustrates results of a typical Western blot and shows that, based on the p24 band intensity, detergent-treated HIV contained substantially less p24 than partially delipidated HIV particles. The p24 content of the samples was also quantified by densitometry analysis of the HIV p24 band. Figure 1B illustrated the densitometry values of the p24 band in the samples. Quantitative analysis of p24 levels using a commercial p24 ELISA (Zeptometrix) was also performed, with the results illustrated in Figure 2. ELISA indicated a significant (~3 log) reduction in p24 levels in detergent-treated HIV as compared to control, which is two orders of magnitude higher than the reduction in p24 levels in partially-delipidated HIV particles as compared to control. These experimental results show that, with respect to preservation of p24 content, partial delipidation of HIV by an organic solvent is unexpectedly advantageous as compared to detergent treatment. Comparison of p24 levels among the samples indicates better preservation of structural integrity of partially delipidated HIV particles as compared to detergent-treated HIV.

Figure 1A: Western blot assay

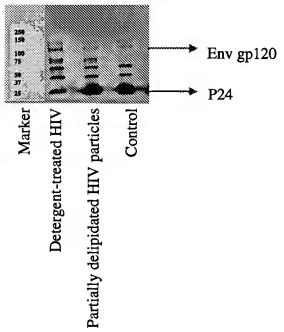


Figure 1B: Densitometry analysis of p24 band intensity in western blot assay

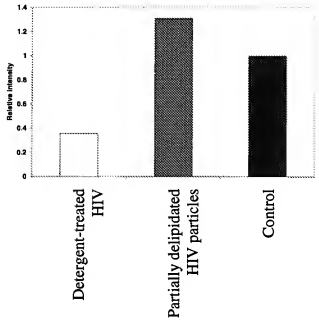
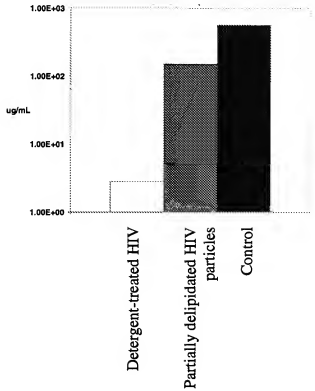
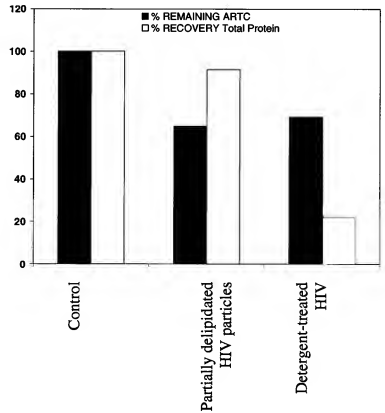


Figure 2: Comparison of p24 levels using quantitative p24 ELISA



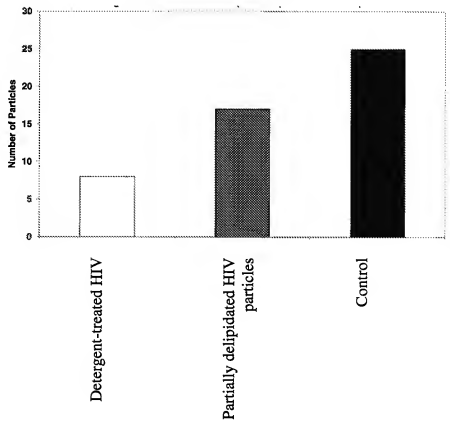
7. Experimental results provided herein show that total protein content is significantly lower in detergent-treated HIV as compared to partially-delipidated HIV particles. Total protein recovery and reduction in cholesterol levels in detergent-treated HIV, partially delipidated HIV particles and control HIV is illustrated in Figure 3. A cholesterol to total protein ratio in partially delipidated HIV particles in decreased as compared to control. In contrast, a cholesterol to total protein ratio in detergent-treated HIV is increased as compared to control. These experimental results also show that partial delipidation of HIV by an organic solvent is unexpectedly advantageous as compared to HIV detergent treatment with regard to preservation of total protein content, which indicates better preservation of structural integrity of partially delipidated HIV particles as compared to detergent-treated HIV.

Figure 3: Comparison of total protein and total cholesterol (ARTC) levels



8. Experimental results provided herein show that detergent-treatment of HIV, as compared to partial delipidation of HIV by an organic solvent, such as DIPE, results in a substantially lower number of virion particles observed by EM. Briefly, the number of HIV particles in 10 different fields (at a direct magnification of 97,000X), was evaluated. Each EM field had an average area of 21487 μm^2. Viral particle counts were performed by an EM expert in a blind procedure. Figure 4 illustrates the results of virion particle counts. These experimental results show that detergent treatment of HIV deleteriously affects structural integrity of the resulting product, detergent-treated HIV. In comparison, partial delipidation by an organic solvent is unexpectedly advantageous relative to detergent treatment because it preserves structural integrity of partially delipidated HIV particles.

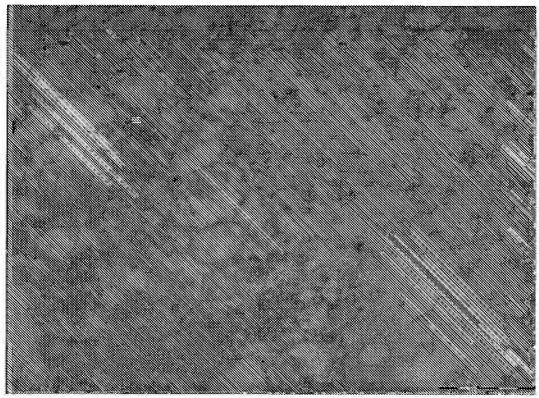
Figure 4: HIV virion particle counts per EM field



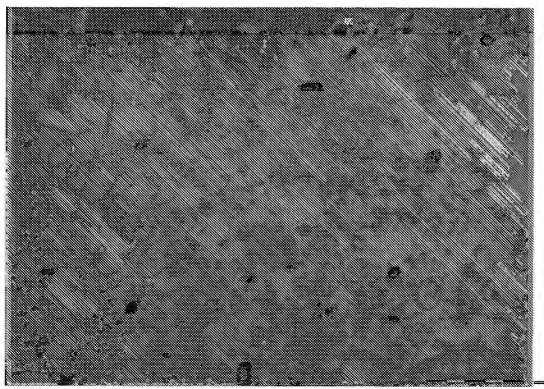
9. Experimental results provided herein show that detergent treatment results in substantial loss of virion structural integrity, as observed by EM. In Figure 5-7, examples of EM images show structures of control HIV, detergent-treated HIV and partially delipidated HIV particles, respectively. Figures 5A, 6A and 7A show EM photomicrographs at 23,000X magnification. The red dots in the photomicrographs indicate virion particles. Figures 5B, 6B and 7B show EM photomicrographs at 97,000X magnification and illustrate virion morphology and virion diameters in each sample. For comparison, Figure 8 shows a schematic illustration and an example of a high resolution EM photomicrographs of an HIV virion (from http://www.klinikum.uniheidelberg.de/uploads/RTEmaciC_fig1_01.jpg.jpg).

Control virus particles shown in Figure 5A have a viral envelope and a center core of HIV capsid (p24). Figure 5B depicts a typical virion of a control mature HIV virion. Figures 6A and 6B depict detergent-treated HIV. Figure 6B shows detergent-treated structurally damaged virions exhibiting poorly delineated envelope, core/capsid, and membrane definition. Figures 7A and Figure 7B depict partially delipidated HIV particles. Figure 7A is visually similar to Figure 5A showing control HIV and is visually distinct from Figure 6A showing detergent-treated HIV. Figure 7B depicting partially delipidated HIV particles shows virion morphology similar to control HIV, as shown in Figure 5B. These experimental results indicated that detergent treatment substantially alters the morphology of HIV virion particles, resulting in stripping of viral envelopes and substantial loss of the viral capsid core. In comparison to detergent treatment, as disclosed in Barrett, partial delipidation by an organic solvent is unexpectedly advantageous for preservation of structural integrity of partially delipidated HIV particles.

Figure 5A: EM photographs of control HIV; Red Dots indicate particles counted, 23 000X magnification

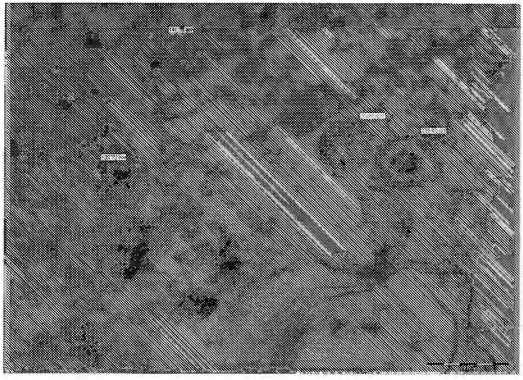


Field 1

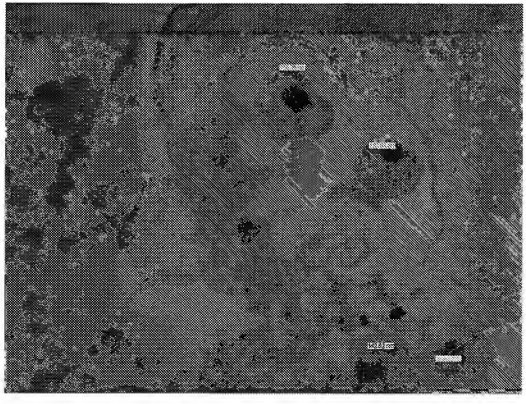


Field 2

Figure 5B: EM photographs of control HIV,97 000X magnification

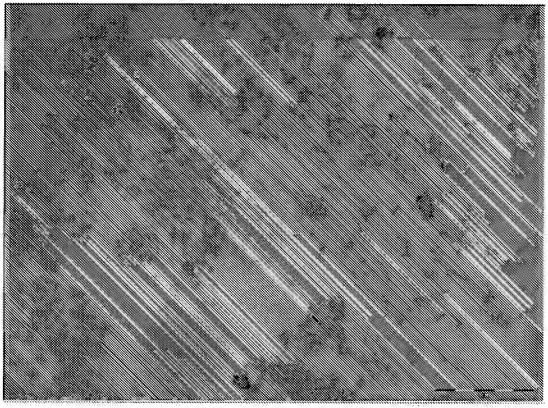


Field 1

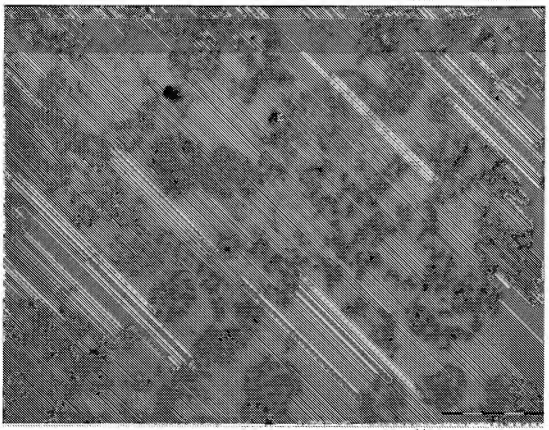


Field 2

Figure 6A: EM photographs of detergent-treated HIV; red dots indicate particles counted, 23 000X magnification



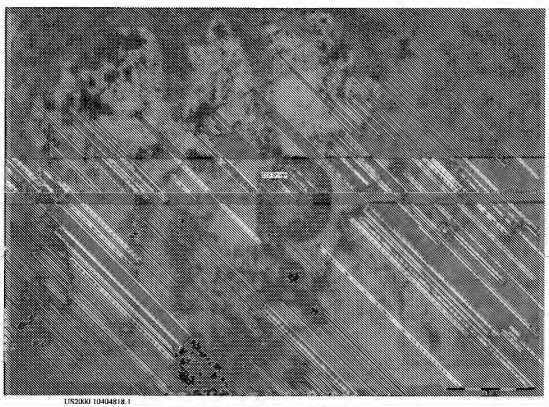
Field 1



Field 2

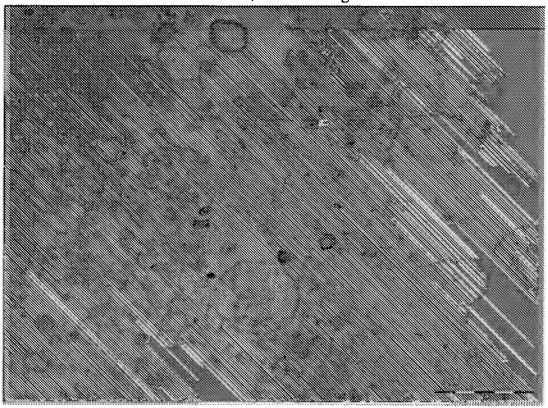
Figure 6B: EM photographs of detergent-treated HIV, 97 000X magnification

Field 1

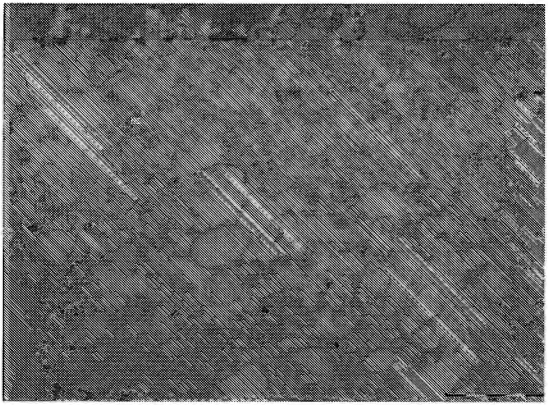


Field 2

Figure 7A: EM photographs of partially delipidated HIV particles; red dots indicate particles counted, 23 000X magnification

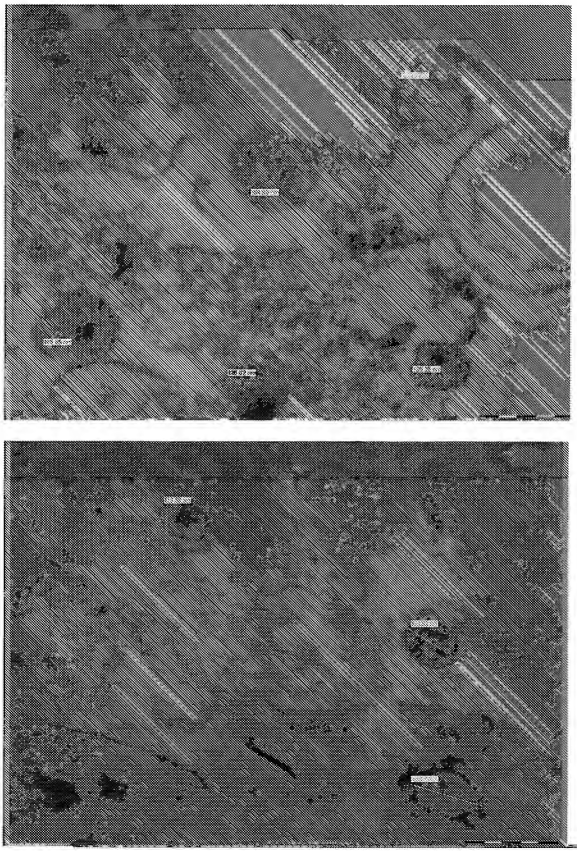


Field 1



Field 2

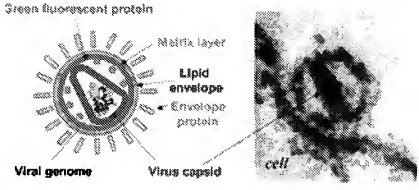
Figure 7B: EM photographs of partially delipidated HIV particles, 97 000X magnification



Field 1

Field 2

Figure 8. A schematic illustration and an example of a high resolution EM picture of HIV virion (from http://www.klinikum.uniheidelberg.de/uploads/RTEmaciC_fig1_01.jpg.jpg).



8. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of any patent issuing on this application.

Moiz Kitabwalla
Signature

Moiz Kitabwalla
Name

October 26th, 2007.
Date

MOIZ KITABWALLA

6619 Tiffany Cmn

Livermore, CA 94551

work-925-249-4011, home-925-209-1081

Email: mkitabwalla@lipidsciences.com

Professional Profile

- Experienced Ph.D. with over nine years of extensive training (academia and industry) in virology, molecular biology, immunology, and biochemistry.
- An innovative, independent researcher highly appreciated by colleagues as an invaluable team contributor.
- Lead researcher, organized all aspects of HIV-1 vaccine pre-clinical trial involving cohorts of non-human primates.
- Highly motivated, analytical, with a reputation for being a catalyst in developing strong and successful collaborative relationships with colleagues in diverse areas of research.
- Excellent communication, organizational, and computer skills, with proven record of aiding N.I.H. funding for laboratories.
- Excellent technical writing skills.

Professional and Research Experience

Lipid Sciences, Inc: Pleasanton, CA. 2003-
Manager/Senior Manager, Viral Programs.

Manager of HIV and Enveloped virus vaccine development Program/Laboratory Manager.

- Led a research team developing novel therapeutic HIV vaccines based on Lipid Sciences patented delipidation process (pre-clinical data manuscript submitted).
- Wrote protocols, manuscripts, and coordinated pre-clinical research on HIV vaccine development with collaborators at Johns Hopkins Medical School, and Emory University.
- Organized annual Viral Advisory Board (V.A.B.) meetings for Lipid Sciences. Had total confidence of the V.A.B.
- Established an oncology vaccine division at Lipid Sciences, to explore companies novel delipidation process in creating better cancer vaccines.-provisional patent application filed.
- Principal Investigator on a STTR grant awarded by the N.I.H. to study SARS vaccine development.
- Established novel assays for measuring reverse cholesterol transport and phospholipids for the cardiovascular platform at Lipid Sciences, Inc.

Dana-Farber Cancer Institute/Harvard Medical School; Boston, MA. 2001-2003
Post-Doctoral Fellow, Harvard Medical School, the Department of Cancer, Immunology, and AIDS.

Lead Researcher on the Project: Blocking Milk-Borne HIV Clade A Transmission.

- Tested the ability of neutralizing human monoclonal antibodies (nmAbs) in blocking mother-to-child HIV clade A transmission, via breast-milk, in sub-Saharan Africa.
- Conducted research on in vitro neutralization assays using primary HIV clade A isolates from East Africa, for their susceptibility to the nmAbs (published).
- Created a chimeric SHIV-A (SIV/HIV chimera) expressing the HIV clade A envelope, by replacing envelopes from molecular clones of SHIV. The novel SHIV-A readily infects rhesus macaque cells, providing an animal model for studying HIV clade A pathogenesis (manuscript in preparation).
- Preliminary research on cross-clade nmAb protection and SHIV-A led to an N.I.H. grant awarded to Dr. Ruth M. Ruprecht, advisor, Harvard Medical School.
- Developed a novel high throughput non-radioactive cytotoxic T-cell assay to replace the chromium release assay (manuscript and patent in preparation).
- Conducted preliminary siRNA/nmAb synergy therapy experiments. Subsequently, published an editorial on siRNA in the New England Journal of Medicine.
- Assisted colleagues in designing experiments to detect in vivo HIV protein-cellular protein interactions using fluorescence resonance emission transfer (FRET).

The Institute of Human Virology (Dr. Robert C. Gallo, Director);
Baltimore, MD. 2000-2001
Visiting Research Graduate Student

Conducted final thesis experiments, and thesis writing.

- Thesis project, "Chemokine regulation of viral pathogenesis" successfully demonstrated the novel utility of gamma chemokine lymphotactin (ltn) as a powerful mucosal adjuvant in HIV vaccines.

University of Wisconsin-Madison; Madison, WI. 1995-2000
Graduate student, Cellular and Molecular Biology Program

Conducted thesis research: Chemokine regulation of viral pathogenesis.

- Cloned, expressed, and purified the rhesus macaque homolog of the novel gamma chemokine lymphotactin (ltn) expressed in E.Coli and Pichia pastoris system.
- Biochemically characterized ltn activity by calcium flux activity and chemotaxis assay, as well as receptor/ligand binding assays.
- Designed in vivo experiments to show functional activity and chemotaxis of lymphocytes by showing ltn was capable of inducing delayed type hypersensitivity (DTH) responses in rhesus macaques.
- Preliminary data on the functional and biochemical characterization led to a N.I.H. R.O.1 grant awarded to thesis advisor, Dr. C.D. Pauza. Funding supported the thesis project, and led to novel recombinant attenuated SHIVs expressing ltn, RANTES, and MIP-1b.
- Created a highly sensitive novel ELISA to detect human and rhesus ltn, as well as Abs for intracellular ltn staining.

- Created several recombinant SHIVs expressing Itn, inactive Itn, and substantially assisted in creating SHIVs expressing b-chemokines RANTES, and MIP-1b.
- Showed in an in vivo vaccine/challenge model that Itn as well as RANTES could act as mucosal adjuvants in enhancing HIV vaccines (published).
- Thesis also contributed towards understanding the immunology of Itn by demonstrating that its expression had a strict requirement for T-cell receptor signalling (published).
- Set up a successful collaboration to elucidate the solution structure of Itn by NMR (published by Dr. Brian Volkman's laboratory). Assisted Dr. Volkman and his team in securing N.I.H. funding for the research.

Abbott Laboratory; Ashland, OH. 1995
Technician, Quality Control-Abbott Plastics and Rubber Division.

Quality control for rubber and plastics components made in the Ashland plant.

- Routinely used GC-mass spectroscopy and HPLC, to monitor quality of rubber and plastic consumables such as baby nipples.

Education

Ph.D. Molecular Biology, University of Wisconsin-Madison, Cellular and Molecular Biology Program, Madison, WI. 2001

- Thesis project- Chemokine Regulation of Viral Pathogenesis- entailed testing the utility of chemokines as mucosal adjuvants for HIV vaccines
- Research involved a cohort of 24 non-human primates, and coordination of experimental protocols, assays, and schedules.

B.Sc. (Summa Cum Laude), Biochemistry/Molecular Biology, University of Wisconsin-Eau Claire, Eau Claire, WI. 1995

- Gained valuable research experience in virology by designing experiments to elucidate anti-TNF mechanisms developed by a human DNA virus, cytomegalovirus (CMV).

Professional Affiliations

American Association of Immunologists (AAI)-Member

The Federation of American Societies for Experimental Biology (FASEB)-Member

MOIZ KITABWALLA

6619 Tiffany Cmn

Livermore, CA 94588

work-925-249-4011, home-925-209-1081

Email: mkitabwalla@lipidsciences.com

Publications

1. **Moiz Kitabwalla**, Francois Villinger, Aftab A. Ansari, James E.K. Hildreth, Hassibullah Akeefe, Zhaohao Liao, Ann E. Mayne, Lisa Gargano, Adam P. Conner, Jo-Ann Maltais, Gretchen Kunas, and Marc Bellotti. Enhancement of cell mediated immune responses using lipid depleted lentivirus as immunogen: A novel approach for inducing recognition of new viral epitopes. Accepted. Vaccine.

2. Paul M. Waterman*, **Moiz Kitabwalla***, Glen S. Hatfield, Peter S. Evans, Yichen Lu, Ilia Tikhonov, Joseph M. Bryant, and C. David Pauza. Effects of virus burden and chemokine expression on immunity to SHIV in nonhuman primates. Viral Immunology. In Press. 2004 *Authors contributed equally to the research.

3. Jeffrey Safrit, Ruth Ruprecht, Flavia Ferrantelli, Weidong Xu, **Moiz Kitabwalla,** Koen Van Rompay, Marta Marthas, Nancy Haigwood, John Mascola, Katherine Lazuriaga, Samuel A. Jones, Bonnie J. Mathieson, and Marie-Louise Newell; Ghent IAS Working Group on HIV in women children. Journal of Acquired Immune Deficiency Syndrome. (2).pp169-77, 2004.

4. Flavia Ferrantelli, **Moiz Kitabwalla**, Robert A. Rasmussen, Chuanhai Cao, Ting-Chao Chou, Hermann Katinger, Gabriela Stiegler, Lisa A. Cavacini, Yun Bai, Joseph Cotropia, Kenneth E. Ugen, and Ruth M. Ruprecht. Potent cross-group neutralization of primary Human Immunodeficiency Virus isolates with Monoclonal antibodies-Implications for Acquired Immunodeficiency Syndrome vaccine. Journal of Infectious Diseases. (1).pp71-74, 2004.

5. Ruth M.Ruprecht, Flavia Ferrantelli, **Moiz Kitabwalla,** Weidong Xu, and Harold M.McClure. Antibody protection:Passive immunization of neonates against oral AIDS virus challenge. Vaccine. (21).pp3370-3373, 2003.

6. **Moiz Kitabwalla**, Flavia Ferrantelli, Tao Wang, Alistair Chalmers, Hermann Katinger, Gabriela Stiegler, Lisa A. Cavacini, Ting-Chao Chou, and Ruth M. Ruprecht. Primary African HIV Clade A and D Isolates: Effective Cross-Clade Neutralization with a Quadruple Combination of Human Monoclonal Antibodies Raised Against Clade B. AIDS Research and Human Retroviruses. (19), No:2.pp125-131, 2003.

7. Paul M. Waterman*, **Moiz Kitabwalla***, Ilia Tikhonov, and C. David Pauza. Simian/Human Immunodeficiency Virus89.6 Expressing the Chemokine Genes MIP-1β, RANTES, or Lymphotactin. Viral Immunology. (16), No:1.pp35-44, 2003. *Authors contributed equally to the research.

8. **Moiz Kitabwalla**, and Ruth M. Ruprecht. RNA Interference: a new weapon against HIV and beyond. New England Journal of Medicine. (347), No:17.pp 1364-1367, 2002.

9. **Moiz Kitabwalla,** Tao Wang, Jeremy McKay, Julie Overbaugh, and Ruth M. Ruprecht. Construction and Characterization of a Replication Competent Simian/Human Immunodeficiency Virus (SHIV) Expressing the HIV-1 Clade A Envelope (SHIV-A). In Preparation.

10. **Moiz Kitabwalla,** Alistair Chalmers, Tao Wang, Robert Rasmussen, Shisong Jiang, Jeremy McKay, and Ruth M. Ruprecht. A high through-put assay that effectively replaces the classic ^{51}chromium release assay for measuring CTL and ADCC activity. In Preparation.

11. **Moiz Kitabwalla**, Alistair Chalmers, Robert Rasmussen, Shisong Jiang, Tao Wang, Jeremy McKay, and Ruth M. Ruprecht. A novel method for measuring CTL and NK cell-mediated cytotoxicity against targets infected by viruses, using two-color flow cytometry. In Preparation.

12. Ilia Tikhonov, **Moiz Kitabwalla**, Miroslav Malkovsky, Marianne Wallace, and C. David Pauza. Staphylococcal superantigens induce lymphotactin production by human CD4+ and CD8+ T cells. Cytokine. (16), no: 2, pp73-78, 2001.

13. Sonay Kuloglu, D.R. McCaslin, **Moiz Kitabwalla**, C. David Pauza, John L. Markley, and Brian F. Volkman. Monomeric solution structure of the prototypical 'C' chemokine Lymphotactin. Biochemistry (40), no: 42, pp 12486-12496, 2001.

14. Marianne Wallace, Paul M. Waterman, Jacque L. Mitchem, Mahmoud Djavani, Charles Brown, Parul Trivedi, Douglas Horejsh, Marta Dykhuizen, **Moiz Kitabwalla**, and C. David Pauza. Lymphocyte activation during acute simian /human immunodeficiency virus $SHIV_{89.6pd}$ infection in macaques. Journal of Virology (73), no:12.pp 10236-10244, 1999.

15. Daniel N. Streblow, **Moiz Kitabwalla**, Miroslav Malkovsky, and C. David Pauza. Cyclophilin A modulates processing of Human Immunodeficiency Virus type I p55 Gag: mechanism for antiviral effects of cyclosporin A. Virology (245), no:2.pp197-202, 1998.

16. Daniel N. Streblow, **Moiz Kitabwalla**, and C. David Pauza. Gag protein from Human Immunodeficiency Virus type I assembles in the absence of Cyclophilin A. Virology (252), no:1.pp228-234, 1998.

17. Cheryl L. Muller, Jason R.Bever, Mark S. Dordel, **Moiz Kitabwalla**, Theresa M. Reineke, Justin B. Sausker, Troy R. Seehafer, Yu Li, and Jerry Jasinski. Regiochemistry of Intramolecular photocycloaddition of 1,3-Dioxin-4-ones tethered through the ketal carbon. Tetrahedron Letters (50) no:38.pp 8663-8666, 1997.